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# Identification of an Antimicrobial Compound from *Apium graveolens* Seeds (Celery Seeds)

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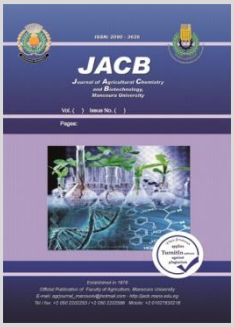


## ABSTRACT

The increasing demand on new antimicrobial compounds is very important nowadays because the microbes become more resistant to the old antimicrobial compounds. Hence, this study was designed to isolate and identify antimicrobial compounds from Celery seeds (*Apium graveolens*). The seeds were extracted by hexane, methylene chloride and methanol (sequentially). Purification was achieved by column chromatography. 1-phenyl-1-hepten-3-ol was isolated from hexane extract and the chemical structure was identified by spectroscopic studies (IR, proton NMR and GC-MS). The hexane extract showed the highest value as antimicrobial activity against *Bacillus cereus*, *Salmonella* sp., *Staphylococcus sp.* and *Penicillium sp*, *Fusarium oxysporum*, *Alterneria solani*, and *Asperglus niger*. However, the extracts (hexane, methylene chloride and methanol) didn't show any inhibition zone against *Pseudomonas sp*.

***Keywords*:** antimicrobial , *Apium graveolens* ,Celery seeds, 1- Phenyl-hepten-3-ol

## INTRODUCTION

Infectious diseases are harmful and cause losses in crop production and inspire disease for human. Due to increasing the resistance of microorganisms for old antibiotics, there are need to discover new antimicrobial compounds from plant, algae.....etc. Herbs and spices are important sources for new antibiotics. Celery (*Apium graveolens*, Apiaceae family) is a native medicinal plant in Europe and found in Algeria, Iran, India, Egypt and USA. Celery (*A. graveolens*) seeds, stem and leaves have several medicinal properties (Kooti *et al*., 2014). *A. graveolens* seeds contain, 5-11% moisture, 0.8% protein, 1.5-3% volatile oil, 5.8-14.2% nonvolatile oil and 6.9-11.0 % total ash (0.5-4.0 % Ash insoluble in acid). Steam distillation for seed produced oil containing 80 % limonene as major constituent (Sahoo *et al*., 2014). Celery plants can preserve several food products because it contains chemical compounds which have antimicrobial properties with low cost and safe (Oussalah *et al.,* 2006). The main objective of this study aim to isolate an antimicrobial compound from *Apium graveolens* seeds (celery seeds) extracts and elucidate chemical structure using spectroscopic analysis (IR, NMR and GC-MS).

## MATERIALS AND METHODS

**1. Material.**

Celery seeds were purchased from local market (Haraz market, Cairo, Egypt) washed with deionized water and dried at 50°C. All solvent and other chemicals used in this work were analytical grade

**2. Microorganisms:**

**Bacterial strains representing Gram positive stain:** *Bacillus cereus* and *Staphylococcus aureus*; besides, Gram negative: *Pseudomonas aeruginosa* and *Salmonella* sp., in addition to four fungal strains: *Alternaria solani*, *Aspergillus niger*, *Penicillium digititum* and *Fusarium oxysporum* were obtained from MERCN, Faculty of Agriculture, Ain Shams University, Egypt. Stock culture were maintained aseptically under optimal conditions for each microorganism and sub-cultured onto nutrient agar and PDA for bacterial and fungal strains, respectively.

**3 Methods.**

**Preparation and extraction of the active compounds from the plant seeds:**

Celery seeds were sequentially soaked in hexane, methylene chloride and methanol. These solvents have polarity indexes of 0, 3.3 and 5.1, respectively. After extraction, each solvent was evaporated to dryness by rotary evaporator at 40 ºC (Martin *et al*., 2014).

**Antimicrobial activity:**

The antimicrobial properties of *A. graveolens* seed extracts were evaluated by diffusion method according to Moosdeen *et al*., (1988) and De Billerbeck (2007) for bacterial and fungal strains, respectively. Bactria were grown on the plate of nutrient agar for 24 h at 37°C, while fungi were grown at PDA for five days at 30°C. After cultivation, bacterial cells and spores were used in inoculation of the medium. Discs of filter paper with 9 mm diameter (sterile blank disc, Whatman) were placed on the agar and 20µl from seeds extract were added on the disc followed by incubation at 37°C for five days. Plates were observed after incubation and inhibition zones were measured. The experiments were replicated three times with duplicate samples.

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**Separation and purification of antimicrobial compounds from hexane extract**

Column chromatography technique was used for purification according to Dey and Harborne (1991)

**A- The hexane extract** (that showed the highest antimicrobial activity) was purified using glass column packed with silica gel 60 (0.063-0.2mm) as stationary phase. The mobile phase used was ethyl acetate: hexane at different portions (150 mL of each) as follows: - ethyl acetate: hexane 5: 95, ethyl acetate: hexane 30: 70, ethyl acetate: hexane 50: 50, ethyl acetate: methanol 50: 50 and methanol alone respectively.

**B- The active fractions obtained from the first column** (fractions No. 4,5, 6) were purified by second column packed with silica gel 60 (0.063-0.2mm) as stationary phase, the mobile phase used was methylene chloride, methanol: methylene chloride 10:90 and methanol, respectively.

**4. Determination of purity of the fractions**

**A- The fraction obtained from column** were applied on TLC plates separately. TLC plates (GF 254, Merk Germany) and the mobile phase was hexane -ethyl acetate (7:3) (Zhou *et al*., 2014).

**B- Gas chromatography** was done in a gas chromatography (hp, model 6890), equipped with a flam ionization detector, manual injection, HP5 fused silica column (5% phenyl-methyl polysiloxane), 30 m , 0.25 mm i.d., film thickness 0.25 mm, and hp ChemStation software system, temperature of oven was adjusted at 60 °C, raising at 3 °C per mint to 250 °C and then held 20 min at 250 °C; injector temperature: 250 °C; carrier gas: helium at 1.0 mL per min; splitting ratio 1: 10; detectors temperature: 300°C (Marongiu *et al.*, 2013).

**5. Identification of the antimicrobial compound:**

**A - GC-MS analysis:**

The antimicrobial compound obtained from hexane extract and purified by silica gel columns was analyzed by GC/MS. The GC was fitted with a quadrupole mass spectrometer, Agilent model 5973 detector. MS conditions were as follows: ionization energy 70 eV, electron impact ion source temperature 200 °C, quadrupole temperature 150 °C, scan rate 3.2 scan per sec. Software adopted to handle mass spectra and chromatograms was a ChemStation and sample was run in methanol. The isolated compound was identified by mass spectra from literature data (Marongiu *et al*., 2013).

**B – IR analysis**

The IR analysis was performed by FTIR (MAC) at Micro Analytical Center, Faculty of Science, Cairo University.

**C- NMR analysis**

The analysis was performed using Bruker NMR spectrometer. $H_1$ spectra were run at 400 MHz in dimethyl sulphoxide (DMSO), the analysis was performed in drug discovery Lab., Faculty of Pharmacy, Ain Shams University.

## RESULTS AND DISCUSSION

**1. Antibacterial activity of *A. graveolens* seed extracts:**

All of the tested extracts (hexane, methylene chloride and methanol) showed inhibition zone against Gram positive bacteria *Bacillus cereus, Staphylococcus aureus* with varied inhibition zone diameters. Hexane extract only showed inhibition of *salmonella sp*. (inhibition zone diameter of 8 mm), while *Pseudomonas auroginosa* was resistant to all the tested extracts (Table 1). Moreover *Bacillus cereus* was the most sensitive bacteria to celery seed extracts. It is clearly shows that celery seed extracts have potential antibacterial property against Gram positive bacteria. These results were in line with the report of Gupta *et al*., (2004) who mentioned that Gram-positive strains showed quicker response in comparison to Gram-negative pathogens when use celery seeds essential oil.

**Table 1. Inhibition zone diameters (mm) of *A. graveolens* seed extracts tested against some bacteria:**

| Bacteria | hexane | methylene chloride | methanol | Means |
|---|---|---|---|---|
| *Bacillus cereus* | 27.0 | 24.0 | 11.0 | 20.67 |
| *Staphylococcus aureus* | 16.0 | 31.0 | 11.0 | 19.34 |
| *Salmonella* sp. | 8 | 0.0 | 0.0 | 2.67 |
| *Pseudomonas auroginosa* | 0.0 | 0.0 | 0.0 | 0.0 |
| Means | 12.75 | 13.75 | 5.5 | |

**2. Antifungal activity of *A. graveolens* seed extracts:** All the tested extracts (hexane, dichloromethane and methanol) showed inhibition against all of the tested fungi with varied inhibition zone diameters, except the methylene chloride and methanol extracts against *Penicillium digitatum*, which inhibited only by the hexane extract (Table 2).

**Table 2. Inhibition zone diameters (mm) of *A. graveolens* seed extracts tested against some fungi:**

| The tested fungi | Hexane extract | methylene chloride | Methanol | Means |
|---|---|---|---|---|
| *A. niger* | 26.0 | 27.0 | 21.0 | 24.67 |
| *A. solani* | 23.0 | 23.0 | 21.0 | 22.34 |
| *F. oxisporum* | 31.0 | 11.0 | 10.0 | 17.34 |
| *Penicillium sp*. | 41.0 | 0.0 | 0.0 | 13.67 |
| Means | 30.25 | 15.25 | 13.0 | |

Among four fungal strains, *A. niger* and *A. solani* were more susceptible to extracts of celery seeds. Among the three used extracts, hexane extract found to be more potent compared to the other two extracts

**3. Purification and antimicrobial activity of *A. graveolens* seed hexane extract:**

The hexane extract that showed the highest antifungal and antibacterial activity was selected for purification and identification as active compounds. The purification was performed by silica gel column eluted with different mobile phases. Fourteen fractions were collected. four fractions from the first elution system, ethyl acetate: hexane mixture (5: 95), Four fractions from the second elution system (ethyl acetate: hexane, 30:70), three fractions from the third elution system (ethyl acetate: hexane 50: 50), one fraction from the fourth elution system (ethyl acetate), one fraction from the fifth elution system (ethyl acetate: methanol mixture 50: 50) and finally one fraction from the sixth elution solvent (methanol). All of the collected fractions were tested against *A. solani* and results showed that fractions number 4, 5, 6 showed inhibition of *A. solani*

**4. TLC analysis of the active fractions:**

The TLC analysis showed that both fractions number 4 and 5 contain the same three compounds, while the fraction number 6 contains two compounds.

**5. Purification of the fractions number 4, 5 and 6:**

Ten sub-fractions were collected from each fraction using the silica gel column. All of the collected sub-fractions were tested against *A. solani*. One sub-fraction from each 4, 5 and 6 fractions showed antifungal activity. The TLC analysis showed one spot, and conform the purity by gas chromatography (GC). The GC analysis showed presence of one compound in the sub-fraction number (4-1), while two

compounds were separated from the sub-fractions number (5-1) and (6-1) (figure 1). So, the sub-fraction number (4-1) was selected for further investigations to identify the chemical structure of the antimicrobial compound.

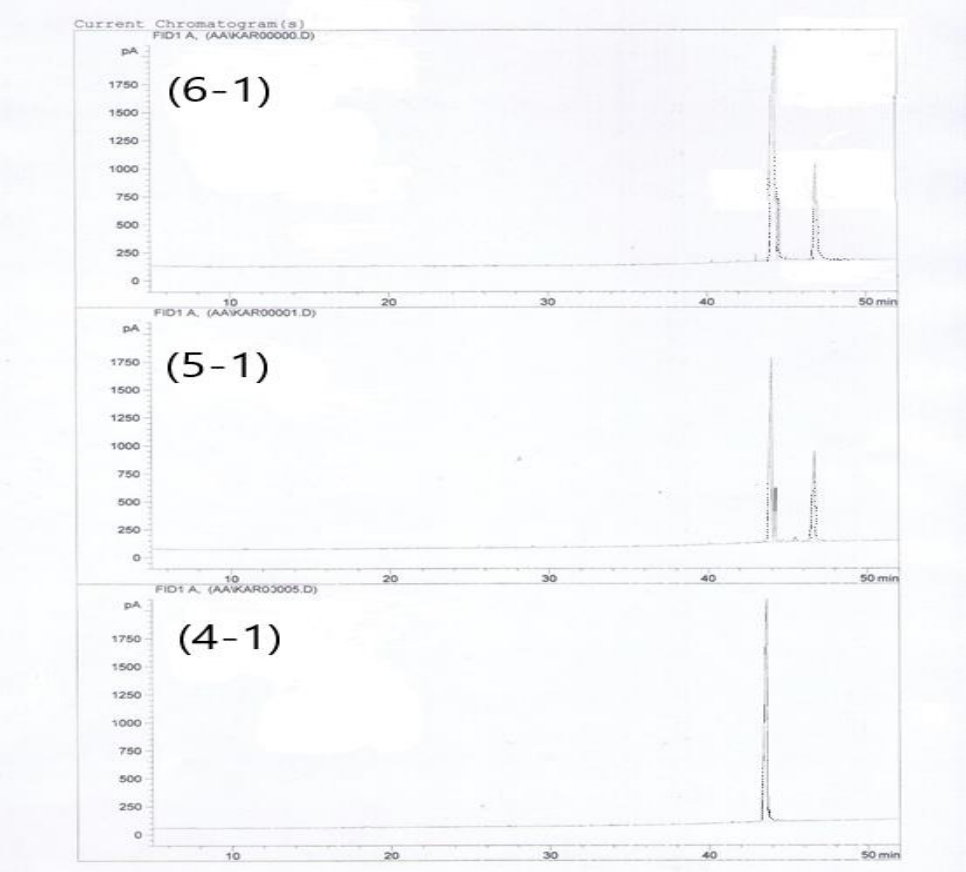



**Fig. 1. GC analysis of the sub-fractions (4-1), (5-1) and (6-1).**

**GC-MS analysis:** GC-MS analysis showed the presence of one compound at retention time 17.65 min. The mass spectrum showed base ion and M+ ion at133.3 m/z and 189.8, respectively, in addition to the fragments 104.9, 147.7, 77, 171.8, 90.8 and 160.8 m/z (Fig. 2). The GC-MS library showed that the molecular formula of active compound $C_{13}H_{18}O$, MW 190. The compound was elucidated as 1-Phenyl-1- hepten-3-ol (fig. 3).

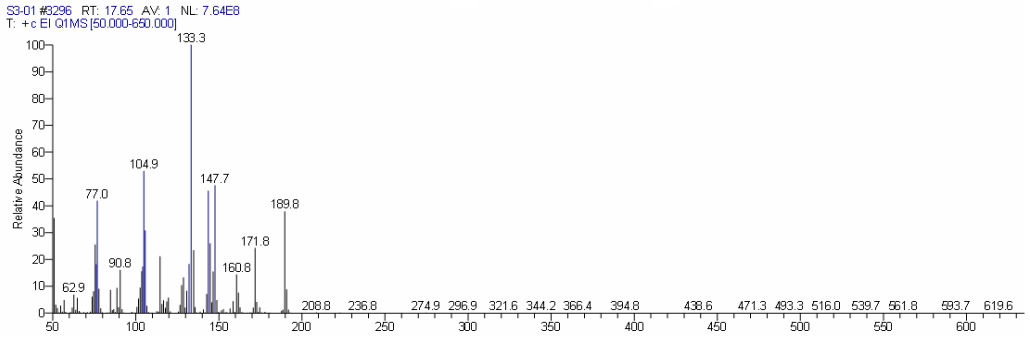



**Fig. 2. The mass spectrum of the pure purified antimicrobial compound**

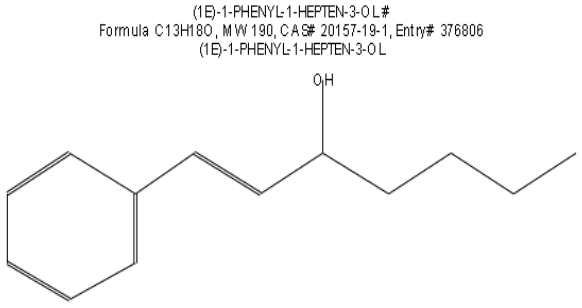



**Fig. 3. 1- Phenyl-hepten-3-ol structure**

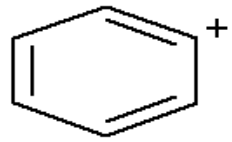
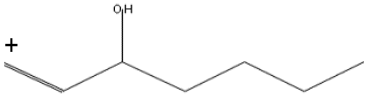
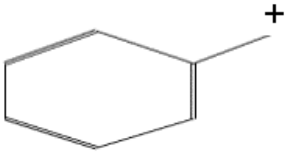
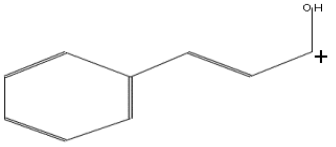
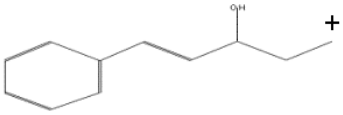
**NMR analysis:**

The NMR spectrum showed aromatic protons at 7-8 ppm, methyl protons at 0.85 ppm, methylene protons at 1.3 ppm, 1.7 ppm and 2.1 ppm, proton is attached to alkene at 5.65 ppm and proton of hydroxyl group at 2.5 ppm (Lonin and Ershov, 1970).

**IR analysis**

The IR analysis showed presence of aliphatic hydrogen at 2964 $cm^{-1}$ and 2897 $cm^{-1}$, hydroxyl group at 3431 $cm^{-1}$, aromatic ring at 1639 $cm^{-1}$ and 1458.8 $cm^{-1}$, C-O at 1018.2 $cm^{-1}$ (Peter, 2011).

**Table. Fragmentation pattern from GC- mass spectra of 1-Phenyl-1- hepten-3-ol**

| Fragmentation pattern | M.W. |
|---|---|
|  | 77 |
|  | 113 |
|  | 90.8 |
|  | 133.3 |
|  | 161 |

## CONCLUSION

Hexane extract of celery seeds has strong antimicrobial activity and one active compound was identified as 1-phenyl-1-hepten-3- ol.

## REFERENCES


De Billerbeck, V.G. (2007). Huiles essentielles et bactéries résistantes aux antibiotiques. Phytothérapie, 5:249-253. https://doi.org/10.1007/s10298-007-0265-z

Dey, P. M.; Harborne J. B. (1991). Terpenoids. In: Charlwood BV, Banthorpe DV, eds. Methods in Plant Biochemistry, New York; Academic Press, (7): 65

Gupta, R., Rath, C.C., Dash, S.K. and Mishra, R.K. (2004). Invitro antibacterial potential assessment of Carrot (*Daucus carota*) and Celery (*Apium graveolens*) seed essential oils against twenty one Bacteria. Journal of Essential Oil Bearing Plants, 7:1, 79-86, https://doi.org/10.1080/0972-060X.2004.10643369

Kooti, W., Sara, A. A., Asadi-Samani, M., Ghadary, H and Ashtary-Larky, D. (2014). A review on medicinal plant of *Apium graveolens*. Advanced Herbal Medicine, 1 (1): 48-59.

Lonin B. I. and. Ershov B. A. (1970). NMR spectroscopy in organic chemistry. Plenum Press, New York ISBN 978-1-4684-1785-2 (eBook)

Marongiu, B., Piras, A., Porcedda, S., Falconieri, D., Maxia, A., Frau, M.A., Goncalvese, M. J., Cavaleiroe, C and Salgueiroe, L. (2013). Isolation of the volatile fraction from *Apium graveolens* L. (Apiaceae) by supercritical carbon dioxide extraction and hydrodistillation: Chemical composition and antifungal activity. Natural Product Research, 27, (17):1521–1527, http://dx.doi.org/10.1080/14786419.2012.725402

Martin, W. W., Matasyoh, J. C. and Kinyanjui, T. (2014). Antimicrobial activity of solvent extracts from the leaves of Tarchonanthus camphoratus (Asteraceae). Journal of Pharmacognosy and Phytochemistry; 3 (1): 123-127

Moosdeen, F., Williams, J.D. and Secker, A. (1988). Standardization of inoculum size for disc susceptibility testing: a preliminary report of a spectrophotometric method. Journal of Antimicrobial Chemotherapy 21(4), 439-43

Oussalah, M., Caillet, S., Saucier, L. and Lacroix, M. (2006). Antimicrobial effects of selected plant essential oils on the growth of a Pseudomonas putida strain isolated from meat. Meat Science. 73(2), 236–244. http://dx.doi.org/ 10.1016/j.meatsci.2005.11.019.

Peter l. (2011) Infrared and Raman Spectroscopy. Elsevier p 3 ISBN: 978-0-12-386984-5

Sahoo, H. B., DasSantani, D. and Sagar, R. (2014) Chemopreventive potential of *Apium leptophyllum* (Pers.) against DMBA induced skin carcinogenesis model by modulatory influence on biochemical and antioxidant biomarkers in Swiss mice. Indian Journal of Pharmacology. 46(5): 531–537. http://dx.doi.org/10.4103/0253-7613.140587

Zhou, Y., Taylor, B., Smith, T., J., Liu, Z., Clench, M., Davies, N. W. and Rainsford, k. D. (2014). A novel compound from celery seed with a bactericidal effect against helicobacter pylori. The journal of Pharmacy and Pharmacology, 61:1067-1077. http://dx.doi.org/ 10.1211/ jpp/61.08.0011.


## تعريف مركب مضاد للميكروبات من بذور نبات الكرفس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مما لاشك فيه أن زيادة الاحتياج الى مضادات ميكروبية جديدة هو شيء مهم جداً بسبب أن الميكروبات أصبحت مقاومة للمضادات الحيوية القديمة ،وحاليا الدراسات تركز على عزل مضادات ميكروبية جديدة من النباتات والطحالب والفطريات والبكتريا ....الخ . فى هذه الدراسة تم استخلاص بذور الكرفس بواسطه بعض المذيبات مثل الهكسان ثم الميثيلين كلوريد ثم الميثانول بالتتابع. وتم التنقية بواسطه عمود الكروماتوجرافى. تم فصل مركب 1-phenyl-1-hepten-3-ol من مستخلص الهكسان وتم دراسة التركيب الكيماوى بواسطة GC-MS,.Proton NMR, IR ، تم ملاحظة أن المستخلص الهكسانى له نشاط مضاد لـ*Bacillus cereus,* و *Staphylococcus sp.* و *Penicillium sp.* و *Fusarium oxysporum* و *Alterneria solani* و *Aspergllus niger* . بالاضافة الى ذلك وجد أن كل المستخلصات ليس لها نشاط مضاد لـ *Salmonella* sp, *Pseudomonas sp*.